# The histone deacetylase inhibitor valproic acid attenuates phospholipase Cγ2 and IgE-mediated mast cell activation

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**Summary sentence**: VPA inhibits IgE-mediated mast cell activation

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## ABBREVIATIONS

**BMMC:** Bone Marrow-derived Mast Cell**, FcεRI:** Fc epsilon Receptor I, **HAT**: Histone Acetyltransferases, **HDAC:** Histone Deacetylase, **HDACi:** Histone Deacetylase Inhibitor, **PLCγ2:** Phospholipase C gamma 2, **SCF:** Stem Cell Factor, **TNP-BSA**: Trinitrophenol-conjugated Bovine Serum Albumin, **TSA:** Trichostatin A, **VPA**: Valproic Acid

## ABSTRACT

Mast cell activation through the high-affinity IgE receptor (FcεRI) plays a central role in allergic reactions. FcεRI-mediated activation triggers multiple signaling pathways leading to degranulation and synthesis of different inflammatory mediators. IgE-mediated mast cell activation can be modulated by different molecules, including several drugs. Herein, we investigated the immunomodulatory activity of the histone deacetylase inhibitor valproic acid (VPA) on IgE-mediated mast cell activation. To this end, bone marrow-derived mast cells (BMMC) were sensitized with IgE and treated with VPA followed by FcεRI crosslinking. The results indicated that VPA reduced mast cell IgE-dependent degranulation and cytokine release. VPA also induced a significant reduction in the cell surface expression of FcεRI and CD117, but not other mast cell surface molecules. Interestingly, VPA treatment inhibited the phosphorylation of PLCγ2, a key signaling molecule involved in IgE-mediated degranulation and cytokine secretion. However, VPA did not affect the phosphorylation of other key components of the FcεRI signaling pathway, such as Syk, Akt, ERK1/2, or p38. Altogether, our data demonstrate that VPA affects PLCγ2 phosphorylation, which in turn decreases IgE-mediated mast cell activation. These results suggest that VPA might be a key modulator of allergic reactions and might be a promising therapeutic candidate.

## INTRODUCTION

Mast cells have a wide distribution in diverse tissues, including the mucosa, where they display important functions during tissue homeostasis [1]. Mucosal mast cells reside in near contact with the environment and are crucial in the early response to pathogens and in the development of allergic diseases [2], which are a serious and growing health problem worldwide [3].

Mast cells originate from hematopoiesis, and contain abundant cytoplasmic granules filled with different chemical mediators such as histamine, heparin, serotonin, cytokines, growth factors, chymase and tryptase [4]. During allergic reactions, mast cells are activated through the cross-linking of Fc epsilon receptors I (FcεRI) by the interaction of allergens with membrane-bound IgE, leading to the release of granules contents, and *de novo* synthesis and secretion of other inflammatory mediators such as prostaglandins, leukotrienes and cytokines. Thus, the chronic activation of mast cells contributes to the pathophysiology of various allergic diseases through the induction of sustained inflammatory environment leading to tissue damage [5]. Therefore, the development of new therapies that enable the regulation of mast cell activation is a priority in allergic diseases. In this line, several approaches are under investigation, including the targeting of activating mast cell-surface receptors or cell-signaling molecules that participate in FcεRI mediated-activation as well as the engagement of mast cell inhibitory receptors [6].

Valproic acid (VPA) is a short-chain fatty acid which is derived from valeric acid, obtained from root extracts of valerian flower plant (*Valeriana officinalis)*. VPA has a broad spectrum of actions and it is commonly used in anticonvulsant therapy and as a mood stabilizer [7]. Research into the mechanism of action has shown that VPA acts as an histone deacetylase inhibitor (HDACi), reverting epigenetically silenced genes in tumor cells, a property that has been harnessed to design new therapeutic approaches for cancer [8, 9]. Recent studies have demonstrated that, through regulation of key cell signaling molecules, VPA can also modulate the functional activity of innate and adaptive immune cells including macrophages, dendritic cells, neutrophils, NK cells, B and T lymphocytes [10]. Considering that IgE-mediated mast cell activation plays a crucial role in allergic diseases and that VPA interferes with cell signaling in several cells of the immune response, this study was conducted to investigate whether VPA could modulate the FcεRI-mediated mast cell activation.

## MATERIAL AND METHODS

### Valproic acid

An injectable solution of sodium valproic acid (Depakene®) was obtained from Hospira Inc. (Pfizer; Kansas, USA).

### Cell culture

Bone marrow-derived mast cells (BMMC) are commonly obtained by culturing with combinations of IL-3 and SCF. However, as reports have shown that chronic exposure to SCF results in attenuated FcεRI signaling and degranulation of mast cells, here we have used cultures of bone marrow from 6-8 week-old C57BL/6 female mice in the presence of IL-3 alone, as described [11, 12]. Mast cell purity was ≥95% as determined by flow cytometry after staining of CD117 (clone: 2B8, BioLegend, USA) and FcεRI (clone: MAR-1, BioLegend, USA). The protocol to derive mast cells from mouse bone marrow was reviewed and approved by the Committee for Ethics in Research, ENCB-IPN.

### Cell viability

BMMC viability was assessed by flow cytometry following staining of cells with Annexin V and propidium iodide. Briefly, BMMC were incubated with VPA for 18 h, and stained with APC-Annexin V (BioLegend, San Diego, CA, USA) and propidium

iodide (eBioscience, Waltham, MA, USA). Viable cells were considered as double negative for both markers.

**IgE-mediated mast cell activation**

To evaluate mast cell degranulation, we followed previously described methods [13, 14]. Briefly, $1x10^6$ BMMC were sensitized with 2 µg/ml monoclonal IgE $\alpha$-trinitrophenyl (clone: MEA-36, BioLegend, San Diego, CA, USA) for 18 h at 37˚C. For VPA treatment, cells were incubated with VPA during sensitization. Then, BMMCs were washed and activated with 100 ng/ml TNP-BSA (Santa Cruz Biotechnology, Dallas, TX, USA) for 1 hour and then stained with anti-CD107a (clone: 1D4B BioLegend, USA).

**Cytokine quantification**

BMMC were cultured with or without 2mM VPA during the sensitization period. Cells were then stimulated with TNP-BSA and supernatants were collected after 24 h of culture. Release of IL-6, TNF-α (BioLegend, San Diego, CA, USA) and IL-13 (eBioscience, Waltham, MA, USA) was measured by ELISA according to the manufacturer's instructions.

**Assessment of mast cell surface receptors expression**

BMMC were incubated with 1 mM VPA for 24 h and then stained with anti- FcεRI (BioLegend, San Diego, CA, USA), anti-CD45 (clone: 30-F11, BD Biosciences, Franklin Lanes, NJ, USA), anti-CD44 (clone: IM7, eBioscience, Waltham, NJ, USA) and anti-CD117 (BioLegend, San Diego, CA, USA).

**Evaluation of phosphorylated proteins**

BMMC were sensitized with IgE, incubated with or without VPA for 18 h, and then activated with 100 ng/mL TNP-BSA. After 5 min of activation, cells were preserved with Fixation Buffer (BD Biosciences, Franklin Lanes, NJ, USA) and permeabilized with Perm Buffer III (BD Biosciences, Franklin Lanes, NJ, USA) or Perm Buffer IV (BD Biosciences, Franklin Lanes, NJ, USA) for phospho (p)-p38 staining. Subsequently, cells were stained with antibodies to p-ERK1/2 (clone: 20A), p-Syk (clone: I120-722), p-PLC-$\gamma$2 (clone: K86-689.37), p-Akt (clone: M89-61), or isotypes controls. For p-p38 (clone: 36/p38), cells were chased after 10 min, permeabilized with Perm Buffer IV (BD Biosciences, Franklin Lanes, NJ, USA) and stained as described above. All antibodies were from BD Biosciences Franklin Lanes, NJ, USA.

**Flow Cytometry**

All cell samples stained with fluorochrome-conjugated antibodies against cell surface markers and intracellular phosphorylated proteins were acquired using

FACSCalibur or FACS Aria III cytometers (BD Biosciences, Franklin Lanes, NJ, USA) and analyzed with FlowJo 7.1 software.

**Data analysis and statistics**

All data were analyzed using GraphPad Prism V6 Software. Data are presented as mean ± SD of at least 3 independent experiments, unless otherwise stated. Statistical significance was determined using ANOVA with Tukey's Multiple Comparison Test or *t-student* test in the case of two groups. Kruskal-Wallis test with Dunnett's Multiple Comparison test was used with normalized data. A *p*-value $<0.05$ was considered statically significant.

## RESULTS AND DISCUSSION

### Low VPA concentrations do not affect mast cell viability

VPAcan inhibit the activation of several cell populations of the immune response [10]. However, VPA also has cytotoxic effects in some cancer cell lines, a feature that has been considered beneficial in the treatment of cancer [8]. Furthermore,VPAshows a concentration-dependent effect on the growth and cell viability of human mast cell line HMC1.2 [15]. To discard the possibility of any modulatory effect of VPA on mast cells being due to their cytotoxic effect, we established a VPA concentration that did not affect BMMC viability. To this end, BMMC were incubated with VPA for 18 h at concentrations that have been shown an effect on activation in other immune cell populations [16,17]. VPA did not induce a significant change in mast cell size or complexity, except at 5 mM which induced a slight increase in cell size (Supplemental. Fig. 1). BMMC treated with 5 or 10 mM VPA showed a significant decrease in their viability when compared with untreated cells. In contrast, 1 or 2 mM VPA did not significantly affect the viability of BMMC (Figs. 1B and C). These VPA concentrations were used in subsequent experiments.

### VPA diminishes IgE-mediated mast cell degranulation

Previous reports indicate that different HDACi can interfere with IgE-mediated mast cell degranulation [18,19]. To determine the ability of VPA to affect mast cell degranulation, BMMC were treated with VPA, sensitized with IgE for 18 h, and

followed by degranulation induction by FcεRI cross-linking with TNP-BSA. VPA-treated BMMC showed a significant reduction in the percentage of degranulated cells after FcεRI cross-linking as evidenced by the expression of CD107a on the cell surface of BMMC (Figs. 1D and E). Interestingly, VPA alone was unable to induce mast cell degranulation (Supplemental. Fig. 2A). This finding shows that the HDACi VPA is an inhibitor of IgE-mediated mast cell degranulation, like other HDACi such as MGCD0103 and Trichostatin A (TSA) [18,19]. VPA also inhibits degranulation in other immune cells, such as NK cells, in which their cytotoxicity against tumoral cells is restrained.16,20 This reduction in NK cell-mediated cytotoxicity by VPA was associated with the inhibition of STAT3 phosphorylation, a molecule that is key in NK cell signaling [20].

**VPA decreases TNF-α, IL-6, and IL-13 release during Ig-E mediated mast cell activation**

In addition to trigger degranulation, FcεRI cross-linking also promotes mast cell production of allergy-related proinflammatory cytokines such as TNF-α, IL-6, and IL-13, which contribute to the pathogenesis of allergic diseases [5]. Therefore, we investigated whether VPA could affected the release of these cytokines in mast cells activated through FcεRI cross-linking. We observed that while treatment of BMMC with VPA alone, or in the presence of IgE, was unable to induce IL-6 release (Supplemental. Fig. 2B), VPA diminished the secretion of the 3 cytokines after FCεRI cross-linking when compared with untreated BMMC (Figs. 1F–H). These results indicate that VPA can interfere with cytokine production after mast

cell activation through FcεRI, as has been observed with other HDACi, such as sodium butyrate,21 and TSA.19 Furthermore, VPA inhibits cytokine production in several cells of the immune response, such as monocytes, macrophages, dendritic cells, and CD4+ T cells, mainly through interfering with the intracellular cell signaling pathways that leads to cytokine production [10].

**Expression of FcεRI is reduced in VPA-treated mast cells**

VPA inhibits activation of immune cells by down-modulating the expression of activating receptors. For instance, VPA reduces the expression of NKG2D on NK cells [20] scavenger receptors and several TLRs on macrophages [22] and costimulatory molecules on plasmacytoid dendritic cells [23]. Based on these findings, we evaluated whether VPA could affect FcεRI expression on mast cells. We observed that VPA induced an average 80% reduction in the cell surface expression of FcεRI on mast cells (Fig. 2A). To address whether VPA affected other activation receptors on mast cells, we evaluated the expression of CD117, the SCF receptor that is involved in mast cell activation and proliferation [24]. We noticed that VPA also reduced CD117 expression on mast cells, but to a lesser extent (Fig 2B). To discern if the inhibitory effect of VPA was generalized on cell surface receptors, we measured the expression of CD44 and CD45 on mast cells. CD44 is a receptor for hyaluronan and is a crucial molecule in the regulation of proliferation and differentiation of dermal mast cells [25], whereas CD45 is a receptor involved in fine-tuning the signaling pathways leading to mast cell activation [26]. Remarkably, VPA did not to affect the expression of CD44 and

CD45 on mast cells (Figs. 2C and D). Finally, we evaluated the kinetics of FcεRI expression on mast cells after VPA treatment. We observed that VPA treatment induced a reduction in FcεRI cell surface expression, which was significative after 18 h of treatment (Fig. 2E). This result is consistent with a previous report showing that the HDACi TSA also affected FcεRI expression on mast cells depending on the time of exposure [19]. Interestingly, VPA exposure had a differential effect on the expression of other mast cell receptors. This differential effect has also been observed in macrophages exposed to the HDACi TSA, which diminished the expression of IL-12p40 but increased the levels of PAI-1 [27]. Consistent with this, VPA has also shown a differential effect on the expression of receptors in tumor cells, promoting an increase in MICA/B andULBP2, but did not affect the expression of ULBP1, 3, and 4 [28]. Thus, these findings indicate that VPA interferes with the expression of the activating mast cell receptors FcεRI and CD117, whereas the expression of CD44 and CD45 are not affected.

### 3.5 VPA decreases phosphorylated PLCγ 2 in mast cells stimulated via FcεRI

Because VPA diminished the expression of FcεRI, we next evaluated if VPA treatment was affecting the intracellular signaling pathways of this receptor in mast cells. To determine optimal time points for evaluation of the phosphorylation of key molecules in mast cell signaling, a time-course of protein phosphorylation was performed after FcεRI cross-linking (Supplemental. Fig. 3). From this analysis, we decided to analyze protein phosphorylation after 5–10 min of activation. The initial signaling following FcεRI cross-linking is mainly dependent on the activation of the tyrosine kinases Lyn and Syk. This activation results in the phosphorylation of Syk,

which is a critical step for the downstream propagation of signals [29]. Unexpectedly, Syk phosphorylation was unaffected by VPA in FcεRI-activated mast cells (Fig. 3A). Next, we evaluated the effect of VPA on phosphorylation of the serine threonine kinase AKT, which is an important signaling molecule for cytokine production during IgE-mediated mast cell activation [30]. We noticed that VPA did not affect the levels of p-Akt when compared with untreated mast cells (Fig. 3B). Besides, the MAP kinase pathway also plays a crucial role during IgE-mediated cytokine production by mast cells [31]. Thus, for a general overview of the MAPK pathway, we evaluated the effect of VPA on the phosphorylation of ERK1/2 and p38 in IgE-activated mast cells. We observed that neither the ERK1/2 nor the p38 phosphorylation status were affected by VPA treatment (Figs. 3C and D).

FcεRI-dependent mast cell degranulation and cytokine secretion are also regulated by complementary signaling pathways, 1 of which involves phospholipase Cγ2 (PLCγ2) [32]. Interestingly, mast cells treated with VPA showed a significant reduction in phosphorylated PLCγ2 when compared with untreated cells (Fig. 3E). Remarkably, previous reports have demonstrated that PLC-γ2-deficient mast cells show a reduction in their degranulation, but the cell signaling through ERK, JNK, and p38 are unaltered [32], as we also observed in our model with VPA. Taken as a whole, our results indicate that VPA diminishes IgE-mediated mast cell degranulation and cytokine secretion by impairing the cell signaling mediated by PLCγ2.

These results show that the immunomodulatory ability of VPA can be extended to mast cells, regulating the IgE-mediated activation of this cell population. Interestingly, although VPA was able to downmodulate the expression of cell surface activating receptors on mast cells, in particular Fc$\varepsilon$RI, this event did not affect the early cell signaling pathway triggered by the cross-linking of the bound IgE in mast cells, indicating that the few Fc$\varepsilon$RI expressed on the mast cell surface were still able to start signaling pathways leading to mast cell activation. Furthermore, the immunomodulatory effect of VPA was associated with a diminished activation of PLC-$\gamma$2 as evidenced by its decreased phosphorylation state. This result reinforces the notion that VPA has the ability to interfere with the activation of key molecules during cell signaling of immune cells. For instance, VPA interferes with the phosphorylation of MDM2, Akt, PI3K, and NF-$\kappa$B in macrophages activated with LPS, whereas in NK cells, VPA reduces STAT3 and STAT5 phosphorylation [10].

To our knowledge, this is the first evidence of PLC$\gamma$2 targeting by VPA in immune cells. PLC usually hydrolyzes phosphatidylinositol (4,5)-bisphosphate into diacylglycerol and inositol 1,4,5-triphosphate, which is crucial for the activation of PKC and the increase of intracellular Ca2+.33 PLC$\gamma$ has 2 isoforms, PLC-$\gamma$1, which is expressed in several cell lineages, and PLC$\gamma$2, which is expressed in hematopoietic cells [33]. PLC$\gamma$2 plays a critical role during the activation of several cells of the immune response including mast cells, macrophages, NK cells, neutrophils, and B lymphocytes [33]. So, it is tempting to speculate that alterations on the activation of these cell

populations by VPA could also be related to the inhibition of PLC$\gamma$2, although further studies are needed.

The mechanism by which VPA alters the cell signaling of immune cells is unclear; although previous reports indicate that VPA attenuates phospholipid cell signaling by an unknown mechanism [34]. It is hypothesized that some HDACi could affect cell signaling through the epigenetic regulation of key molecules [19]. Because of its action as histone deacetylase inhibitor, VPA is able to increase acetylation of histones H2, H3, and H4, leading to modifications in gene expression [35]. This effect has been suggested for TSA, which attenuates IgE-mediated mast cell activation by increasing the expression I-$\kappa$B$\alpha$, the inhibitor of NF-$\kappa$B [19]. A second possibility is that alteration in HDAC function by VPA can induce a change in the acetylation pattern of PLC$\gamma$2, which in turn would alter its ability to be activated through phosphorylation. In this sense, it has been shown that protein acetylation through lysine residues plays a crucial role in different cellular functions, beyond epigenetic regulation [36]. For instance, STAT proteins, which are crucial elements in the cell signaling of several cytokines, are tightly regulated by lysine acetylation, affecting their phosphorylation and biologic function. Interestingly, STAT acetylation also depends on the regulation of histone acetytransferases, HDAC, and sirtuins [37]. Whether PLC$\gamma$2 is susceptible to be modified through acetylation is unknown, although in silico analysis with GPS-PAIL predicts 11 potential acetylation sites (Supplemental. Table 1) [38]. Further work is needed to ascertain whether lysine acetylation plays a role during PLC$\gamma$2 activation.

Beyond its role in allergic reactions, mast cells also play a crucial role in the innate immune response against virus, bacteria, fungi, protozoa, and helminths. Being some of the first cells to sense the presence of pathogens their activation leads to the induction of inflammation and triggering of microbicidal mechanisms that help to contain infection [2,39]. Whether this function is affected by VPA needs to be analyzed. Previous reports indicated that VPA can alter the response of macrophages to bacterial pathogens [10,40]. TLRs plays a crucial role in sensing of different bacteria, and interestingly PLC$\gamma$2 regulates the signaling of TLR-4 [41], suggesting a possible effect of VPA in TLR activation pathway.

In conclusion, our results demonstrate a new effect of VPA, which attenuates mast cell activation through Fc$\varepsilon$RI by inhibiting the activation of PLC$\gamma$2. We suggest that VPA could have a potential therapeutic effect in allergic diseases, but further work is needed to evaluate this possibility.

## AUTORSHIP

GMR-L and RS-C contributed equally to this work. GMR-L, RSC and MC-N performed experiments and analyzed data. GMR-L, RSC, MC-N, SMP-T, FF-B, IW-B, SM-C, RL-S, IEG, SE-P, ADC-B, RC-S analyzed, interpreted data, drafted the manuscript. ADC-B and RC-S designed, supervised the study and obtained funding. All authors critically revised and approved the final version of this manuscript.

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## DISCLOSURE

The authors declare no conflicts of interest.

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## Figure legends

**FIGURE 1** Valproic acid decreases IgE-mediated mast cell activation. (A) Bone marrow-derived mast cell purity as assessed by Fc$\varepsilon$RI and CD117 expression. (B) Representative dot-plots of BMMC exposed for 18 h with different doses of VPA and stained with Annexin V and propidium ioidide (PI). Bottom right panel shows a positive control for cell death induction by heat. (C) Percentage of viable BMMCs exposed to different concentrations of VPA for 18 h. (D) Effect of VPA on mast cell degranulation after Fc$\varepsilon$RI cross-linking. Values were normalized to the maximum response observed in stimulated cells not treated with VPA and set as 1. (E) Representative flow cytometry contour plots of BMMC, gated on FCSH CD107a+ cells. BMMC were sensitized with anti-TNP ($\alpha$-TNP) IgE and treated with either 1 or 2mMVPA for 18 h. Mast cell degranulation was induced with TNP-BSA and cells stained with anti-CD107a. Kruskal–Wallis test with Dunnett's Multiple Comparison Test. *P < 0.05, **P < 0.01,***P < 0.001, n = 4–5.BMMCwere sensitized with anti-TNP ($\alpha$-TNP) IgE and treated with either 1 or 2mMVPA for 18 h. Mast cells were activated with TNP-BSA. After 24 h, cell supernatants were collected and subjected to ELISA for detection of (F) TNF-$\alpha$, (G) IL-6, and (H) IL-13. Data are shown as mean ± SD. ANOVA with Tukey's Multiple Comparison Test. *P < 0.05, **P < 0.01, ***P < 0.001, n = 3

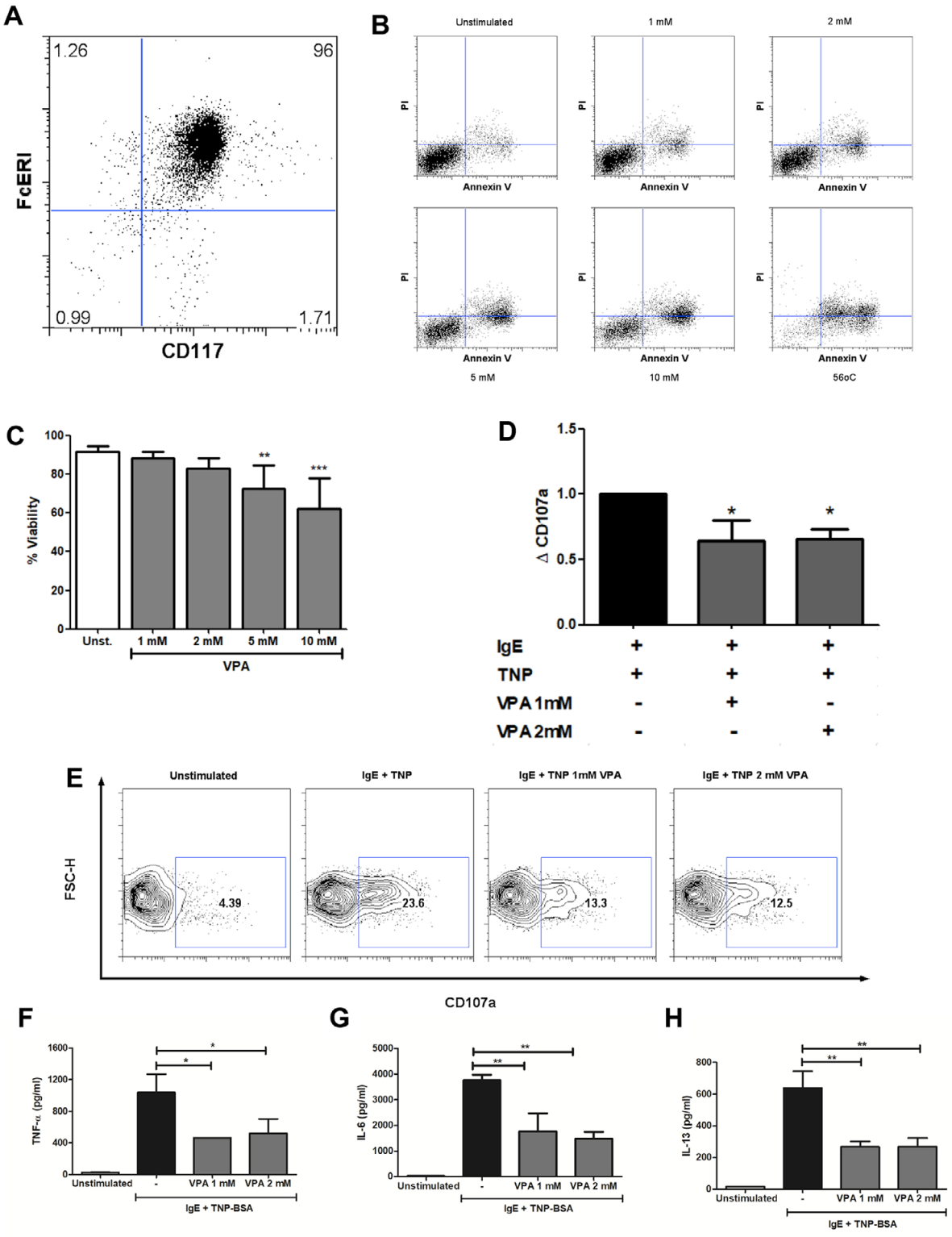
**FIGURE 2** Valproic acid differentially modulates the expression of mast cell surface molecules. BMMC were incubated with VPA during 24 h and the cell surface expression of (A) Fc$\varepsilon$RI, (B) CD117, (C) CD44, and (D) CD45, was measured by

flow cytometry. Left panel, graphs representing the mean fluorescence intensity (MFI) of each molecule. Right panel, representative histograms of each cell surface molecule in BMMC. Student t-test. ***P < 0.001, **P < 0.01. (E) Kinetics of Fc$\varepsilon$RI expression on BMMC incubated with VPA evaluated by flow cytometry. Mean fluorescence intensity (MFI) values for unstimulated BMCC were normalized and set as 1. Data are shown as mean ± SD. Kruskal–Wallis test with Dunnett's Multiple Comparison Test. *P < 0.05, n = 3

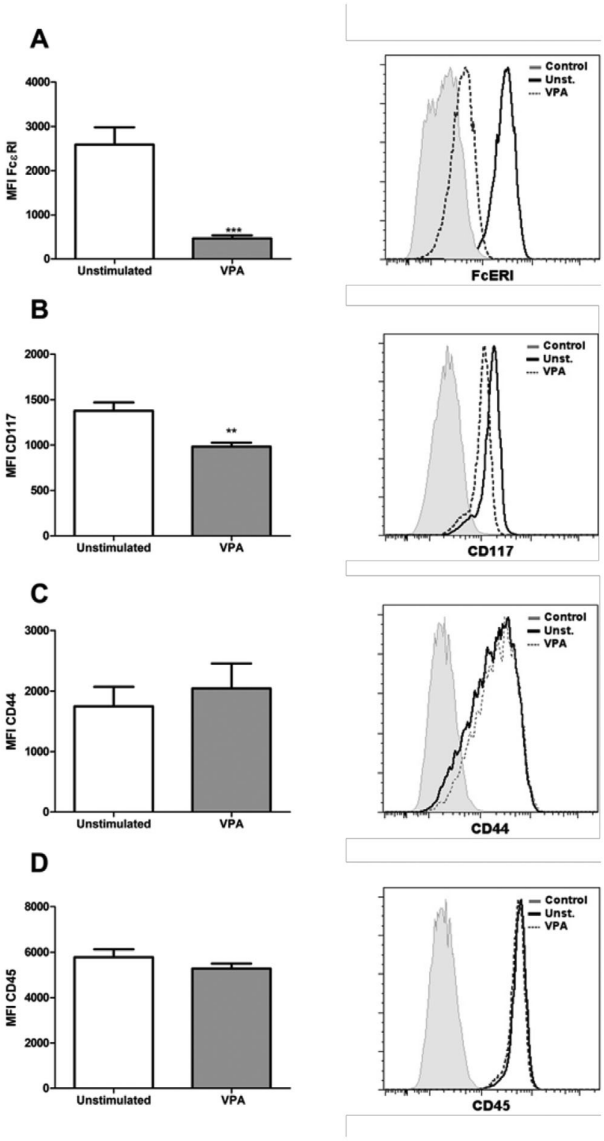
**FIGURE 3** Valproic acid decreases phosphorylated PLC$\boldsymbol{\gamma}$2(pPLC$\boldsymbol{\gamma}$2) on mast cells activated through Fc$\boldsymbol{\varepsilon}$RI. BMMC were sensitized with anti-TNP ($\alpha$-TNP) IgE and treated with 1 mM VPA for 18 h. Fc$\varepsilon$RI was cross-linkedwith TNP-BSA and the phosphorylation state of molecules associated with Fc$\varepsilon$RI-IgE signaling pathway was examined using specific antibodies to (A) p-Syk, (B) p-Akt, (C) p-ERK1/2, (D) p-p38, and (E) p-PLC$\gamma$2 and evaluated by flow cytometry. Left panel, graphs representing the mean fluorescence intensity (MFI) of each phosphoprotein. Right panel, representative histograms of each phosphoprotein in Fc$\varepsilon$RI-activated BMMC. Data are shown as mean ± SD. ANOVA with Tukey's Multiple Comparison Test. ***P < 0.001, n = 3

## FIGURES

### Figure 1



## Figure 2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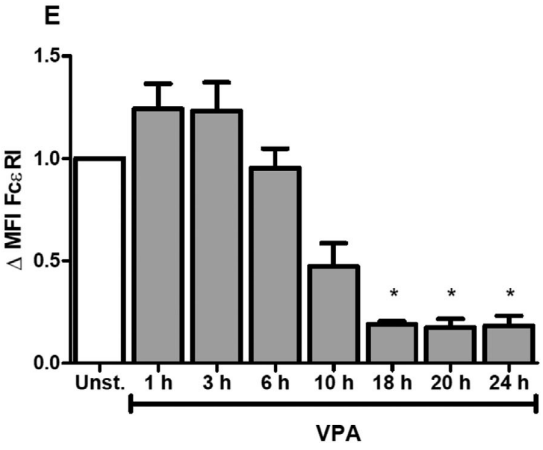
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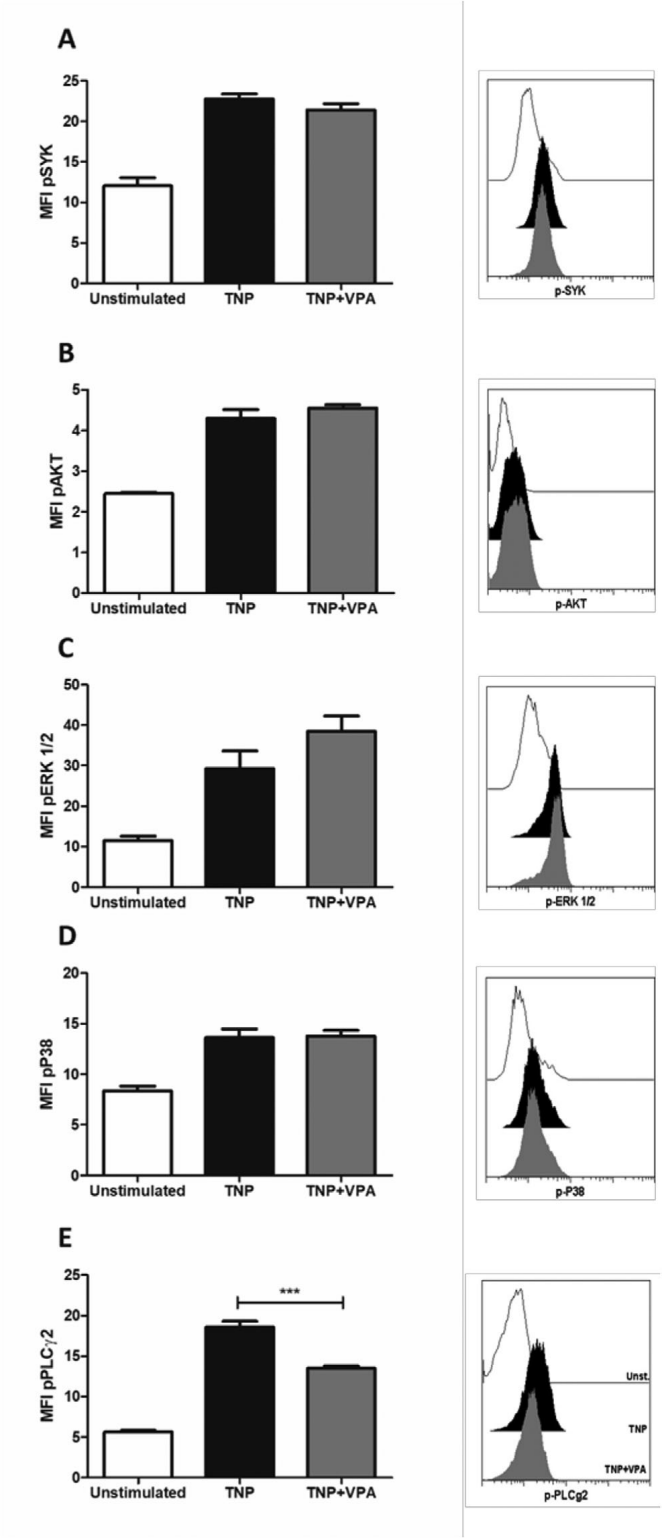
20 h
24 h
VPA

## Figure 3

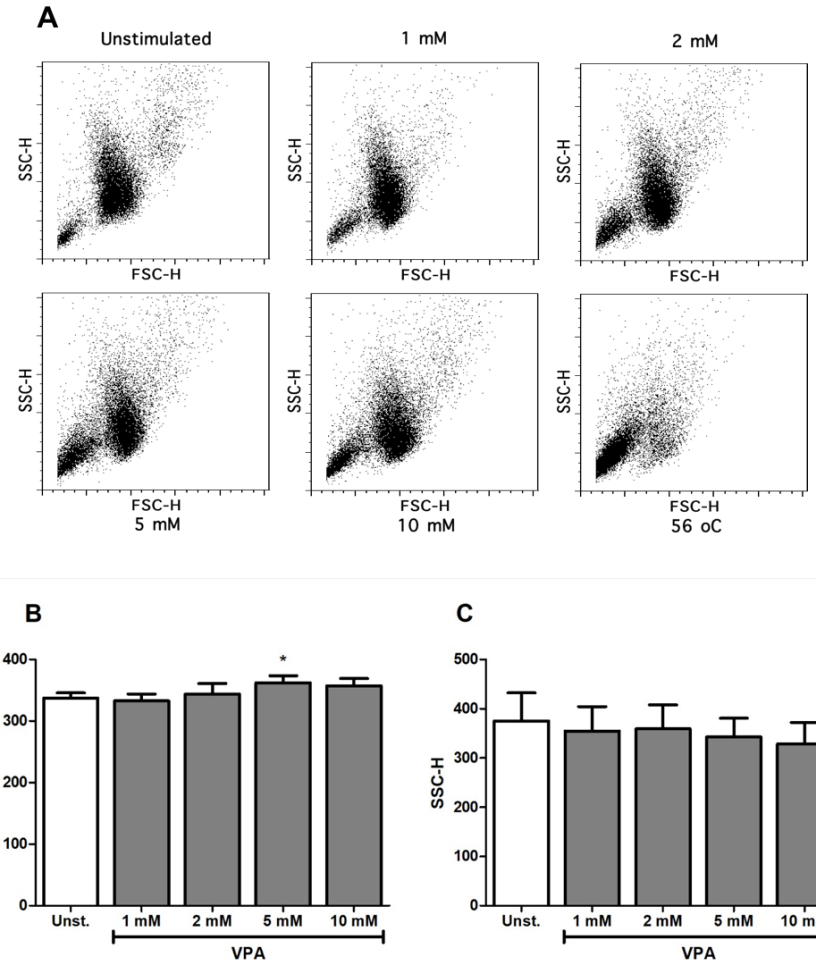



## SUPPLEMENTARY INFORMATION

**Supplementary Figure 1. Valproic acid does not alter mast cell size and complexity**. BMMCs were exposed to different concentrations of VPA for 18 h and analyzed by flow cytometer. A) Representative dot plots of SSC-H and FSC-H are shown. Effect of VPA on B) FSC-H and C) SSC-H of BMMC. Data are shown as mean ± SD. Kruskal-Wallis test with Dunnett's Multiple Comparison Test.

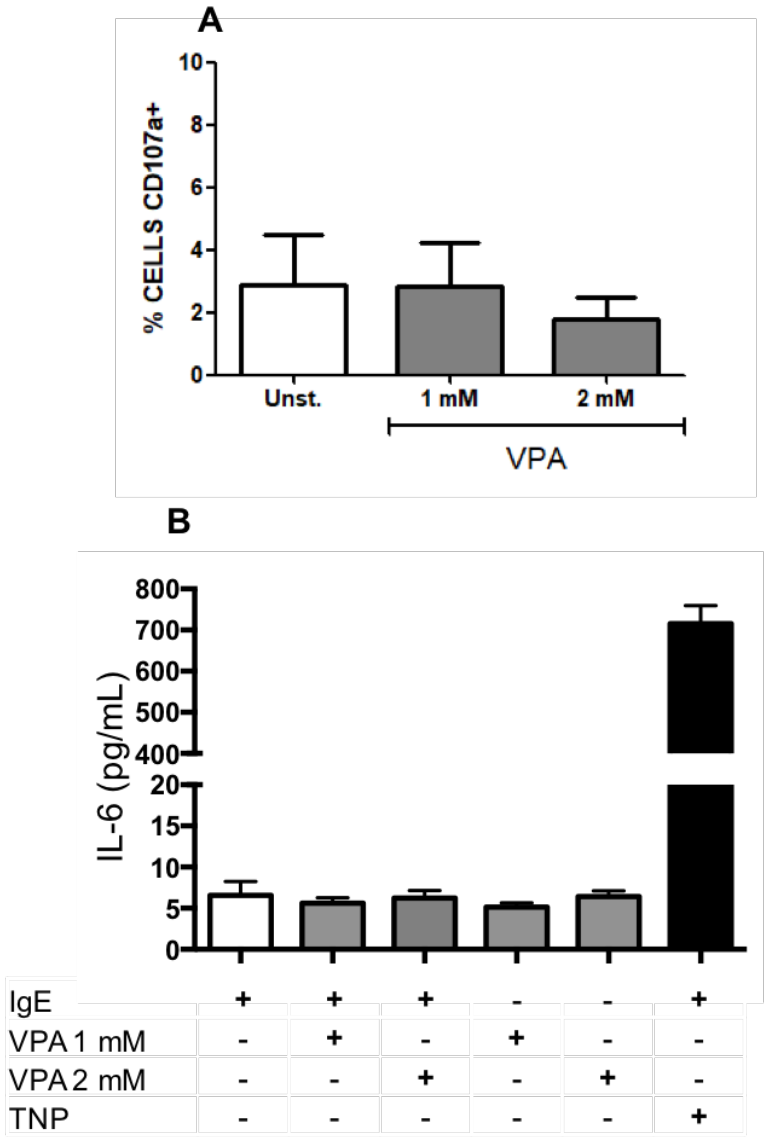
**P < 0.01, * P < 0.05, n = 4.

Supplementary Figure 1



**Supplementary Figure 2. Valproic acid does not activate mast cells**. A) BMMC were cultured with 1 mM or 2 mM VPA during 1 h and stained with anti-CD107a, n = 2. B) BMMC were cultured with VPA alone or sensitized with anti-TNP (α-TNP) IgE in the presence or absence of VPA for 18 h. Cell supernatants were collected and IL-6 was evaluated by ELISA. Data are shown as mean ± SD, n = 4.

Supplementary Figure 2



**Supplementary Figure 3. Kinetic of protein phosphorylation after FcεRI crosslinking in mast cells** . BMMCs were incubated with anti-TNP (α-TNP) during 18 h. FcεRI was crosslinked with TNP-BSA. Protein phosphorylation was evaluated for the indicated times by intracellular staining and Mean Fluorescence Intensity (MFI) is showed for A) phospho-SYK, B) phospho-AKT, C) phospho-ERK1/2, D) phospho-P38 and E) phospho-PLCγ2.

Supplementary Figure 3

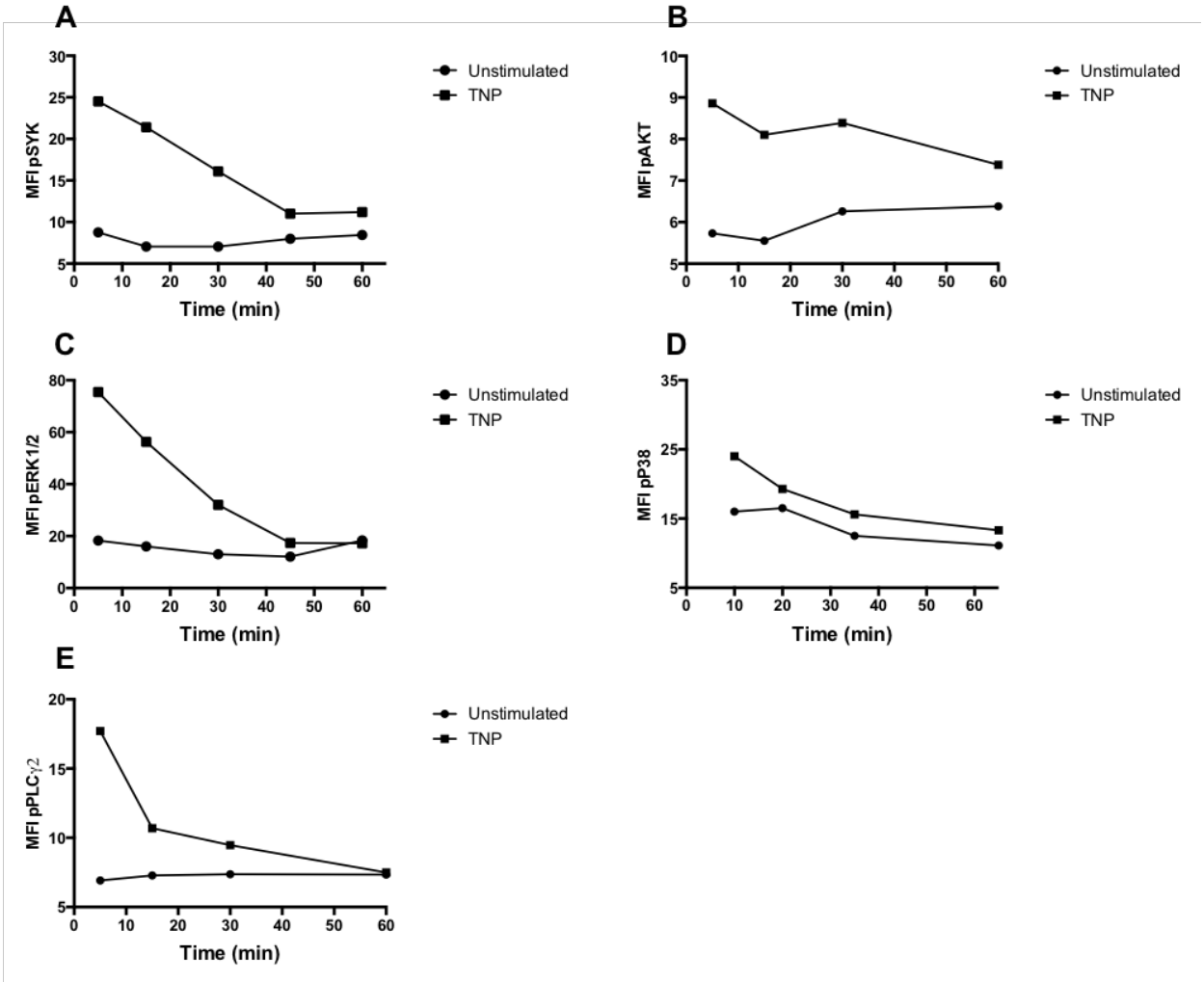


**Table S1**. Prediction of acetylation regions in mouse PLC-γ2 according to GPS-PAIL [37].

| Position | Peptide | HAT |
|---|---|---|
| 15 | DTLPEYEKSQIKRAL | EP300 |
| 86 | SKDFERAKAVRHKAE | KAT2B |
| 91 | RAKAVRHKAECCFTI | KAT2B |
| 419 | EQQRHMAKVFKEVLG | KAT8 |
| 454 | LREKIIIKHKKLGPK | CREBBP |
| 476 | EDKKDEHKPQGELYM | KAT2B |
| 689 | ITFRARGKVKHCRIN | HAT1 |
| 867 | VVKAPQGKNQKAFVF | KAT2B |
| 918 | DTKENNMKYWERNQS | KAT2A |
| 1248 | QLQLYQEKCNRRLRE | CREBBP |
| 1256 | CNRRLREKRVSNSRF | CREBBP |